

WHAT IS CLAIMED IS:

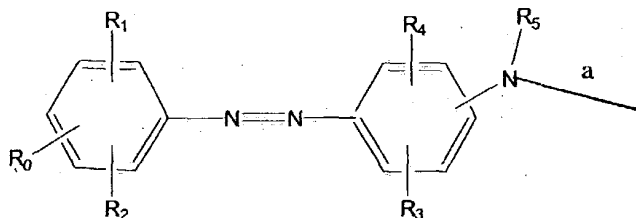
1. An oligonucleotide conjugate having the formula



where ODN is an oligonucleotide or nucleic acid;

FL is a fluorophore moiety covalently attached to the ODN through a linker having the length of 0 to approximately 30 atoms, and

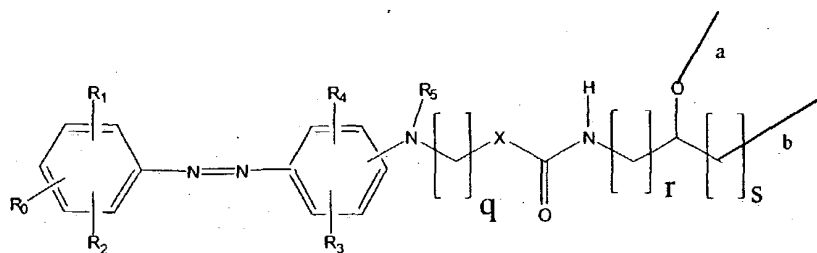
Q is a quencher moiety covalently attached to the ODN through a linker having the length of 0 to approximately 30 atoms, the quencher moiety having the structure



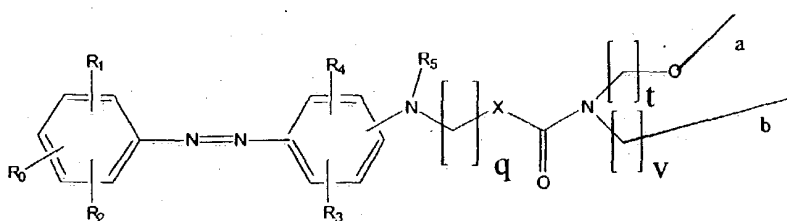
where R₀, R₁, R₂, R₃ and R₄ are independently -H, halogen, -O(CH₂)_nCH₃, -(CH₂)_nCH₃ where n=0 to 5, -NO₂, -SO₃, -N[(CH₂)_nCH₃]₂ where n'=0 to 5 or -CN, and R₅=-H or -(CH₂)_nCH₃ where n''=0 to 5, and where the quencher moiety is attached to the linker through the valence bond designated a.

2. An oligonucleotide conjugate in accordance with Claim 1 where R₀ is H, R₁ is NO₂ in the 4 position of the benzene nucleus, R₂ is H or Cl in the 2 position of the benzene nucleus, and R₃ and R₄ are hydrogen and R₅ is ethyl.

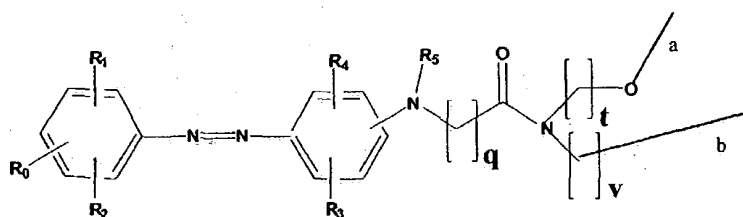
3. An oligonucleotide conjugate in accordance with Claim 1 where the quencher moiety and the linker attaching it to the ODN comprises the structures selected from the moieties shown by the formulas Q-1, Q-2 and Q-3



Q-1



Q-2

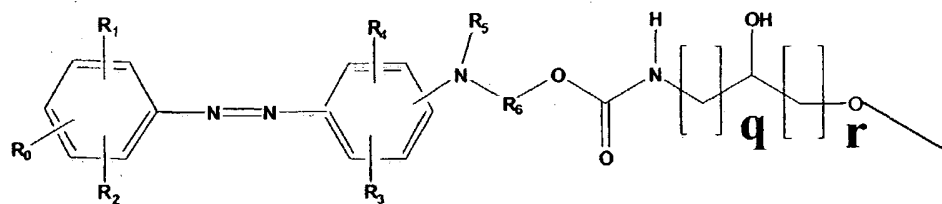


Q-3

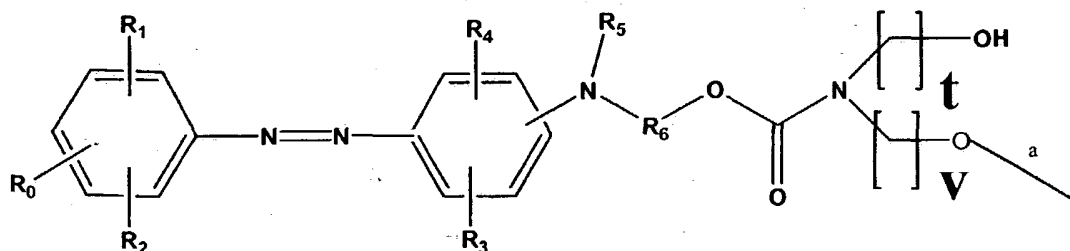
where q is 1 to 20, X is $-O-$, $-OCH_2-$ or $-CH_2-$; t and v independently are 1 to 20, r and s independently are 1 to 20, and the conjugated quencher and linker moiety is attached to the ODN through one of the valence bonds designated a or b .

4. An oligonucleotide conjugate in accordance with Claim 3 further comprising a minor groove binder moiety attached to the quencher-linker conjugate through one of the valence bonds designated a or b .

5. An oligonucleotide conjugate in accordance with Claim 1 where the quencher moiety and of the linker attaching it to the ODN comprises the structures selected from the moieties shown by the formulas Q-4, and Q-5



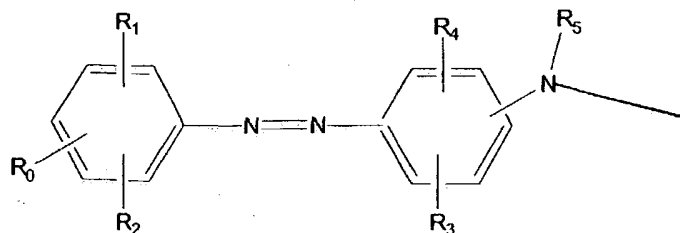
Q-4



Q-5

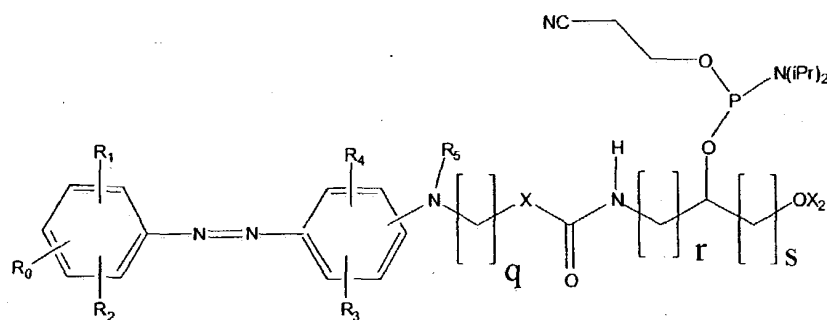
where R_6 is $-(CH_2)_{n^*}$ where n^* is 1 to 20, and t and v independently are 1 to 20, and where the quencher moiety is attached to the ODN through the valence bond designated a .

6. A phosphoramidite reagent for preparing an oligonucleotide-fluorophore-quencher conjugate, the reagent including the moiety

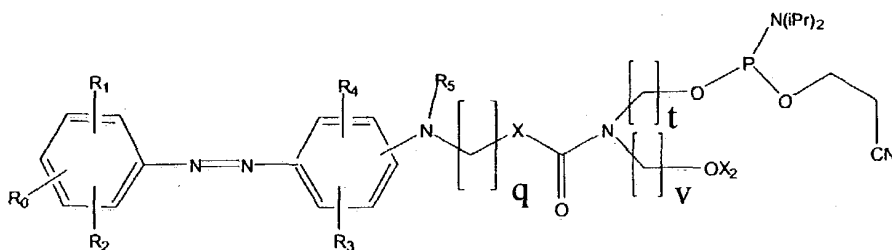


where R_0, R_1, R_2, R_3 and R_4 are independently $-H$, halogen, $-O(CH_2)_nCH_3$, $-(CH_2)_nCH_3$ where $n = 0$ to 5 , $-NO_2$, $-SO_3$, $-N[(CH_2)_nCH_3]_2$ where $n' = 0$ to 5 or $-CN$, and $R_5 = -H$ or $-(CH_2)_nCH_3$ where $n'' = 0$ to 5 , and a bis(methylethyl)amino](2-cyanoethoxy)phosphinoxy moiety covalently linked thereto.

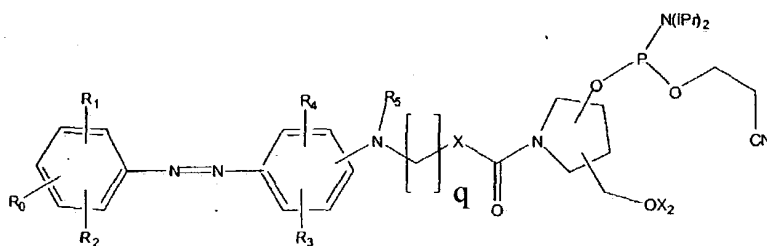
7. A phosphoramidite reagent in accordance with Claim 6 having the formula selected from the group consisting of the formulas designated PA-1, PA-2 and PA-3



PA-1



PA-2



PA-3

where R_0, R_1, R_2, R_3 and R_4 are independently $-H$, halogen, $-O(CH_2)_nCH_3$, $-(CH_2)_nCH_3$ where $n = 0$ to 5 , $-NO_2$, $-SO_3$, $-N[(CH_2)_nCH_3]_2$ where $n' = 0$ to 5 or $-CN$, and $R_5 = -H$ or $-(CH_2)_nCH_3$ where $n'' = 0$ to 5 , q is 1

to 20, X is -O- or -CH₂-; t, v, r and s independently are 1 to 20, and X₂ is H or dimethoxytrityl, methoxytrityl, trityl or an acid labile blocking group.

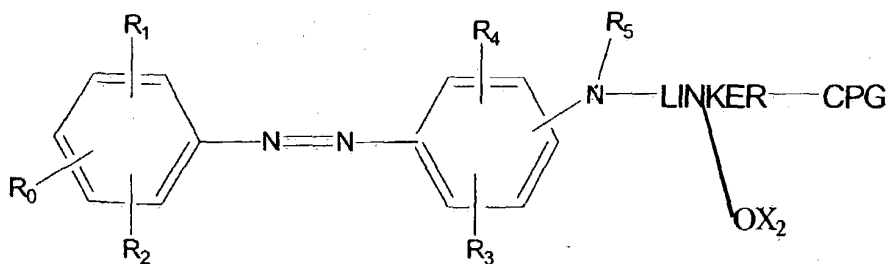
8. A phosphoramidite reagent in accordance with Claim 7 that has the formula designated PA-1.

9. A phosphoramidite reagent in accordance with Claim 7 that has the formula designated PA-2.

10. A phosphoramidite reagent in accordance with Claim 7 that has the formula designated PA-3.

11. A phosphoramidite reagent in accordance with Claim 7 where R₀ is H, R₁ is NO₂ in the 4 position of the benzene nucleus, R₂ is Cl in the 2 position of the benzene nucleus, and R₃ and R₄ are hydrogen and R₅ is ethyl.

12. A covalently linked solid support and quencher conjugate suitable for oligonucleotide synthesis, having the structure



where CPG stands for a polymeric solid support;

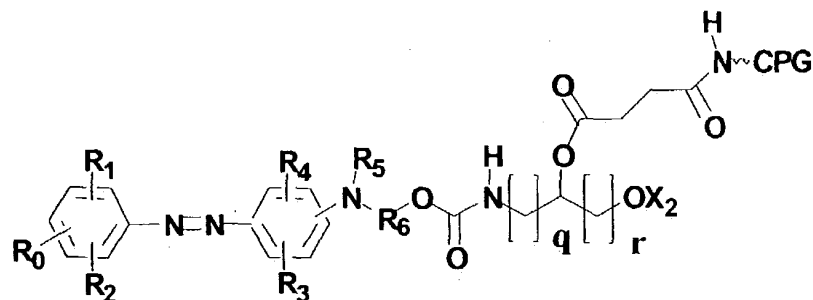
LINKER is a moiety having the length of 1 to approximately 30 atoms and linking the diphenylazo moiety to the CPG;

X₂ is OH or , dimethoxytrityl, methoxytrityl, trityl or an acid labile blocking group;

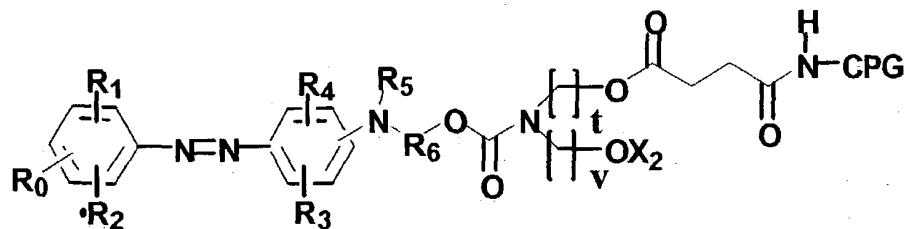
R₀, R₁, R₂, R₃ and R₄ are independently -H, halogen, -O(CH₂)_nCH₃,

$-(CH_2)_nCH_3$ where $n = 0$ to 5 , $-NO_2$, $-SO_3$, $-N[(CH_2)_nCH_3]_2$ where $n' = 0$ to 5 or $-CN$, and $R_5 = -H$ or $-(CH_2)_{n''}CH_3$ where $n'' = 0$ to 5 .

13. A covalently linked solid support and quencher conjugate in accordance with Claim 12 selected from the structures



and



where R_6 is $-(CH_2)_{n^*}$ where n^* is 1 to 20 , and q , r , t and v independently are 1 to 20 .

14. A covalently linked solid support and quencher conjugate in accordance with Claim 13 where R_0 is H , R_1 is NO_2 in the 4 position of the benzene nucleus, R_2 is Cl in the 2 position of the benzene nucleus, and R_5 is ethyl.

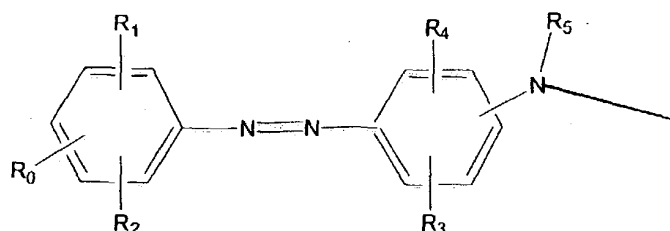
15. An oligonucleotide conjugate having the formula

FL-ODN-Q- MGB

where ODN is an oligonucleotide or nucleic acid;

FL is a fluorophore covalently attached to the ODN through a linker having the length of 0 to approximately 30 atoms, and

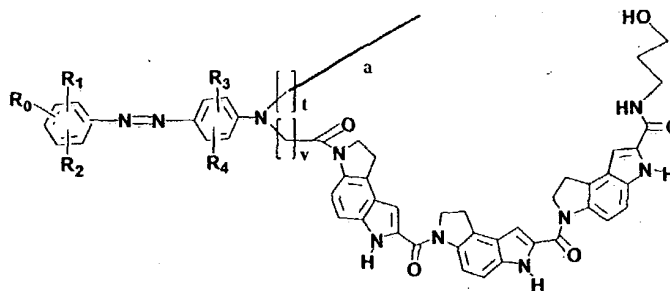
Q is a quencher moiety covalently attached to the ODN through a linker having the length of 0 to approximately 30 atoms, the quencher moiety having the structure



where R_0 , R_1 , R_2 , R_3 and R_4 are independently -H, halogen, $-O(CH_2)_nCH_3$, $-(CH_2)_nCH_3$ where $n = 0$ to 5 , $-NO_2$, $-SO_3$, $-N[(CH_2)_{n'}CH_3]_2$ where $n' = 0$ to 5 or $-CN$, and $R_5 = -H$, $-(CH_2)_{n''}CH_3$ or $-(CH_2)_{n''}$ where $n'' = 0$ to 5 , and

MGB is minor groove binder moiety covalently attached to the ODN moiety or to the quencher moiety through a linker having the length of 0 to approximately 30 atoms.

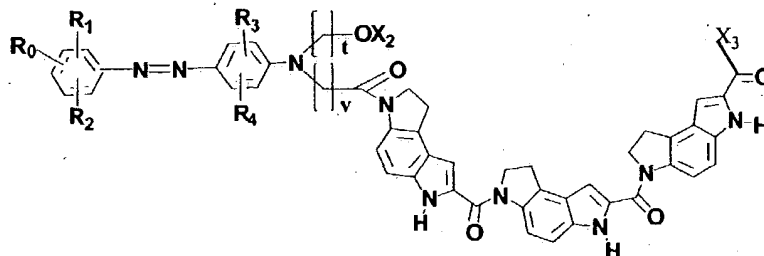
16. An oligonucleotide conjugate in accordance with Claim 15 where the MGB moiety is attached to the quencher moiety, and the covalently bonded MGB-Q moiety has the structure



where t and v independently are 1 to 20, and the valence bond designated **a** attaches the MGB-Q moiety to the ODN moiety.

17. An oligonucleotide conjugate in accordance with Claim 16 where R_0 is H, R_1 is NO_2 in the 4 position of the benzene nucleus, R_2 is H or Cl in the 2 position of the benzene nucleus, and R_3 and R_4 are hydrogen.

18. A covalently bonded minor groove binder and quencher reagent for oligonucleotide synthesis, having the formula



where R_0 , R_1 , R_2 , R_3 and R_4 are independently -H, halogen, $-O(CH_2)_nCH_3$, $-(CH_2)_nCH_3$ where $n=0$ to 5, $-NO_2$, $-SO_3$, $-N[(CH_2)_nCH_3]_2$ where $n'=0$ to 5 or $-CN$, and t and v independently are 1 to 20;

X_2 is H or dimethoxytrityl, methoxytrityl, trityl or an acid labile blocking group, and

X_3 is pentafluorophenyl, or NH-LINKER-CPG or O-LINKER-CPG where CPG is a polymeric solid support and LINKER is a linking moiety having a length of approximately 0 to 30 atoms linking the tricyclic moiety to the CPG.

19. A covalently bonded minor groove binder and quencher reagent in accordance with Claim 18 wherein X_3 is pentafluorophenyl.

20. A covalently bonded minor groove binder and quencher reagent in accordance with Claim 18 wherein X_3 is NH-LINKER-CPG or O-LINKER-CPG.

21. A covalently bonded minor groove binder and quencher reagent in accordance with Claim 18 where R_0 is H, R_1 is NO_2 in the 4 position of the benzene nucleus, R_2 is H or Cl in the 2 position of the benzene nucleus, R_3 and R_4 are hydrogen and $v=t=3$.

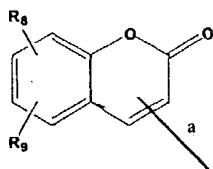
22. An oligonucleotide conjugate having the formula

FL-ODN-Q

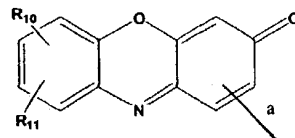
where ODN is an oligonucleotide or nucleic acid;

Q is a quencher moiety covalently attached to the ODN through a linker having the length of 0 to approximately 30 atoms, and

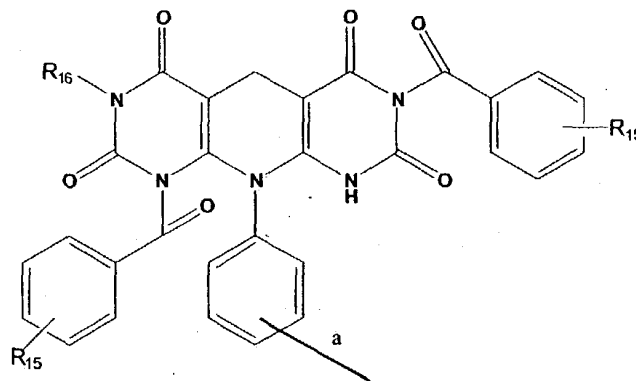
FL is a fluorophore covalently attached to the ODN through a linker having the length of 0 to approximately 30 atoms, said fluorophore moiety having the structure selected from the group designated FL-1, FL-2 and FL-3,



FL-1



FL-2



FL-3

wherein R_8 and R_9 independently are H, halogen, $-\text{NO}_2$, $-\text{SO}_3$, $-\text{C}(=\text{O})\text{NH}_2$, or $-\text{CN}$; $-\text{OR}_{nn}$, $-\text{SR}_{nn}$, $-\text{OR}_{nn}$, $-\text{NHR}_{nn}$, $-\text{N}[\text{R}_{nn}]_2$ where R_{nn} is independently H, an alkyl group of 1 to 10 carbons or an alkanoyl group of 1 to 10 carbons;

R_{10} and R_{11} independently are H, $-\text{CN}$, $-\text{OR}_{12}$, $-\text{N}(\text{R}_{12})_2$, halogen, $-\text{O}(\text{CH}_2)_n\text{CH}_3$, $-(\text{CH}_2)_n\text{CH}_3$, $-\text{NO}_2$, $-\text{SO}_3$, $-\text{C}(=\text{O})\text{NH}_2$, $-\text{N}[(\text{CH}_2)_n\text{CH}_3]_2$ where $n=0$ to 5, or R_{12} is alkyl of 1 to 10 carbons alkanoyl of 1 to 10 carbons,;

R_{15} is H or alkyl of 1 to 10 carbons;

R_{16} is alkyl of 1 to 10 carbons, and

the valence bond designated a symbolizes covalent attachment of the fluorophore to the linker.

23. An oligonucleotide conjugate in accordance with Claim 22 where the fluorophore has the formula designated FL-1.

24. An oligonucleotide conjugate in accordance with Claim 23 where R_8 is $OC(O)CH(CH_3)_2$ and R_9 is H.

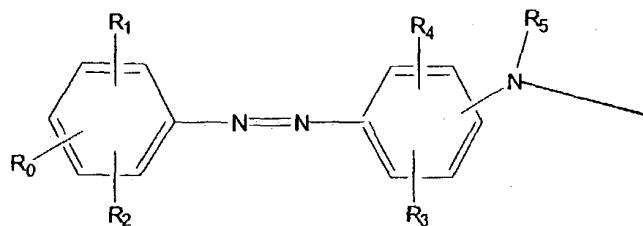
25. An oligonucleotide conjugate in accordance with Claim 22 where the fluorophore has the formula designated FL-2.

26. An oligonucleotide conjugate in accordance with Claim 25 where R_{10} is $OC(O)CH(CH_3)_2$ and R_{11} is H.

27. An oligonucleotide conjugate in accordance with Claim 22 where the fluorophore has the formula designated FL-3.

28. An oligonucleotide conjugate in accordance with Claim 28 where R_{15} is methyl and R_{16} is *n*-propyl.

29. An oligonucleotide conjugate in accordance with Claim 22 where the quencher moiety comprises the structure

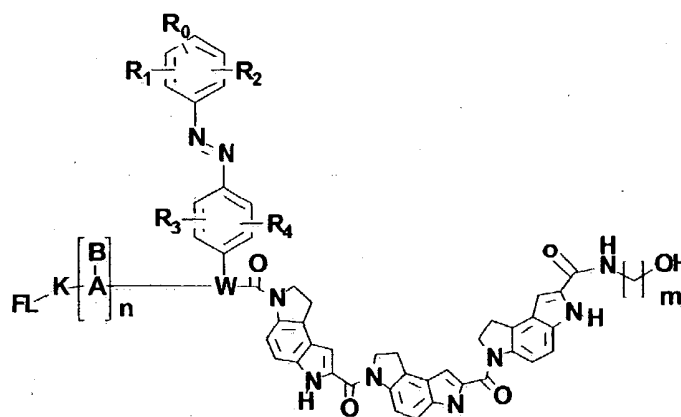


where R_0 , R_1 , R_2 , R_3 and R_4 are independently -H, halogen, $-O(CH_2)_nCH_3$, $-(CH_2)_nCH_3$ where $n = 0$ to 5, $-NO_2$, $-SO_3$, $-N[(CH_2)_nCH_3]_2$ where $n' = 0$ to 5 or $-CN$, and $R_5 = -H$ or $-(CH_2)_nCH_3$ where $n'' = 0$ to 5.

30. An oligonucleotide conjugate in accordance with Claim 22 comprising an additional minor groove binder moiety (MGB) attached to the quencher moiety through a linker having the length of 0 to approximately 30 atoms, whereby the oligonucleotide conjugate has the formula



31. An oligonucleotide conjugate of the formula



wherein R_0 , R_1 , R_2 , R_3 and R_4 are independently -H, halogen, $-O(CH_2)_{n^*}CH_3$, $-(CH_2)_{n^*}CH_3$ where $n^*=0$ to 5, $-NO_2$, $-SO_3$, $-N[(CH_2)_{n'}CH_3]_2$ where $n'=0$ to 5 or $-CN$;

FL is a fluorophore moiety with emission wavelengths in the range of about 300 to about 800 nm;

K is a linker containing 1 to approximately 30 atoms selected from the group consisting of C, O, N, S, P and H;

$[A-B]_n$ symbolizes an ODN, DNA, RNA or PNA or any combination thereof, where A is the sugar phosphate backbone where the sugar and the phosphate may independently be modified; B is a heterocyclic base, where B is independently selected from purine, pyrimidine, pyrazolo[3,4-d]pyrimidine, 7-substituted pyrazolo[3,4-d]pyrimidine-, 7-deazapurine, 7-substituted 7-

deazapurine, and modified purine- and pyrimidine-bases, and where the DNA, RNA, PNA or ODN can include any combinations of these bases, and

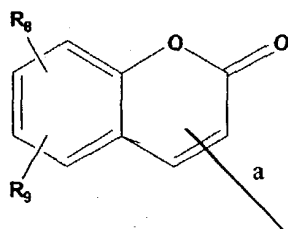
and n is the number of nucleotide units in said DNA, RNA, PNA or ODN;

W is a linker of a length of 0 to approximately 30 atoms, selected from the group consisting of C, O, N, S, P and H, and

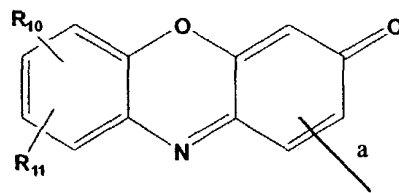
m is an integer having the values of 1 to 20.

32. An oligonucleotide conjugate in accordance with Claim 31 where R_0 is H, R_1 is NO_2 in the 4 position of the benzene nucleus, R_2 is H or Cl in the 2 position of the benzene nucleus, and R_3 and R_4 are hydrogen.

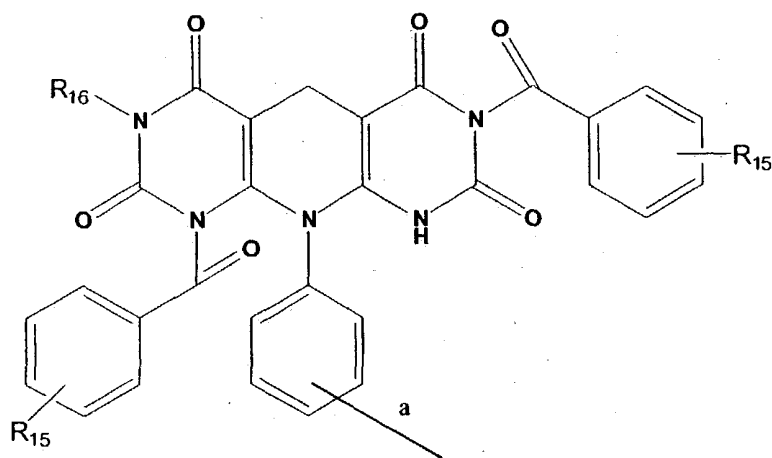
33. An oligonucleotide conjugate in accordance with Claim 31 where said fluorophore moiety has the structure selected from the group designated FL-1, FL-2 and FL-3,



FL-1



FL-2



FL-3

where R_8 is OH or O-alkanoyl where the alkanoyl group has 1 to 10 carbons;

R_9 is H or alkyl of 1 to 10 carbons;

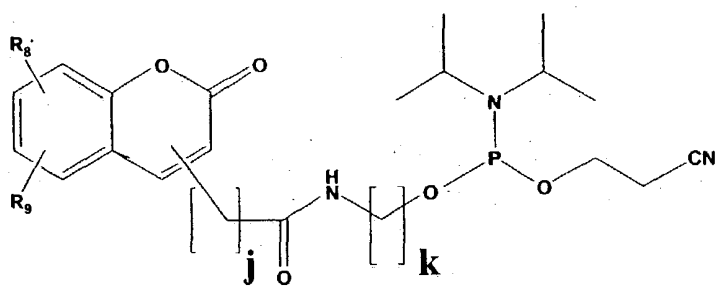
R_{10} and R_{11} independently are H, $-OR_{12}$, $-NHR_{13}$, halogen, $-O(CH_2)_nCH_3$, $-(CH_2)_nCH_3$, $-NO_2$, $-SO_3$, $-C(=O)NH_2$, $-N[(CH_2)_nCH_3]_2$ or $-CN$ where $n=0$ to 5;

R_{15} is H or alkyl of 1 to 10 carbons;

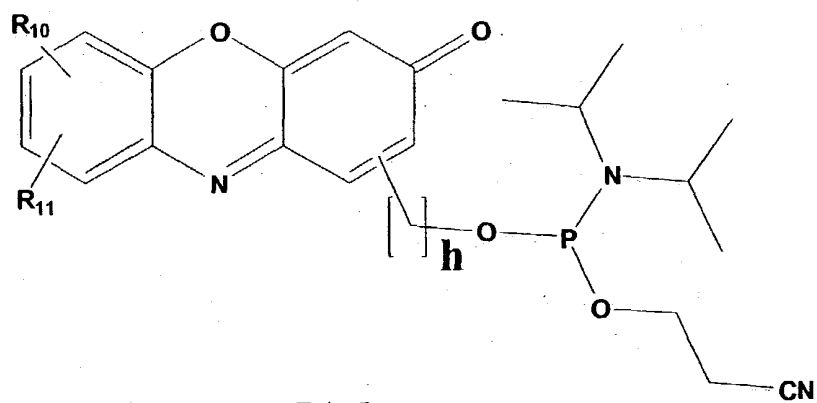
R_{16} is alkyl of 1 to 10 carbons, and

the valence bond designated a symbolizes covalent attachment of the fluorophore to the linker K.

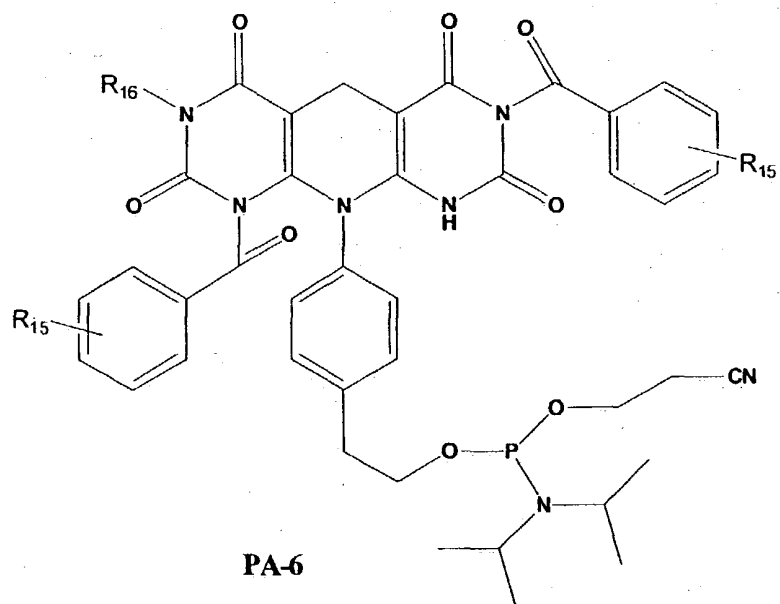
34. A phosphoramidite reagent for preparing an oligonucleotide-fluorophore-quencher conjugate, the reagent selected from the group consisting of the structures designated PA-4, PA-5 and PA-6,



PA-4



PA-5



PA-6

wherein R_8 and R_9 independently are H, halogen, $-\text{NO}_2$, $-\text{SO}_3$, $-\text{C}(=\text{O})\text{NH}_2$, or $-\text{CN}$; $-\text{OR}_{nn}$, $-\text{SR}_{nn}$, $-\text{OR}_{nn}$, $-\text{NHR}_{nn}$, $-\text{N}[\text{R}_{nn}]_2$ where R_{nn} is independently H, a blocking group compatible with oligomer synthesis

removable under acid or alkaline conditions; or an alkyl or alkanoyl group having 1 to 10 carbon atoms;

j and k independently are 1 to 10;

R_{10} and R_{11} independently are H, $-OR_{12}$, $-NHR_{13}$, halogen, $-O(CH_2)_nCH_3$, $-(CH_2)_nCH_3$, $-NO_2$, $-SO_3$, $-C(=O)NH_2$, $-N[(CH_2)_nCH_3]_2$, O-alkyl or O-alkanoyl where the alkanoyl group has 1 to 10 carbons, or $-CN$ where $n=0$ to 5; $h=1$ to 20; and R_{12} and R_{13} are blocking groups compatible with ODN synthesis;

R_{15} is H or alkyl of 1 to 10 carbons;

R_{16} is alkyl of 1 to 10 carbons.

35. A phosphoramidite reagent in accordance with Claim 34 that has the formula designated PA-4.

36. A phosphoramidite reagent in accordance with Claim 35 where R_8 is $-OC(O)CH(CH_3)_2$, R_9 is H, j is 2 and k is 6.

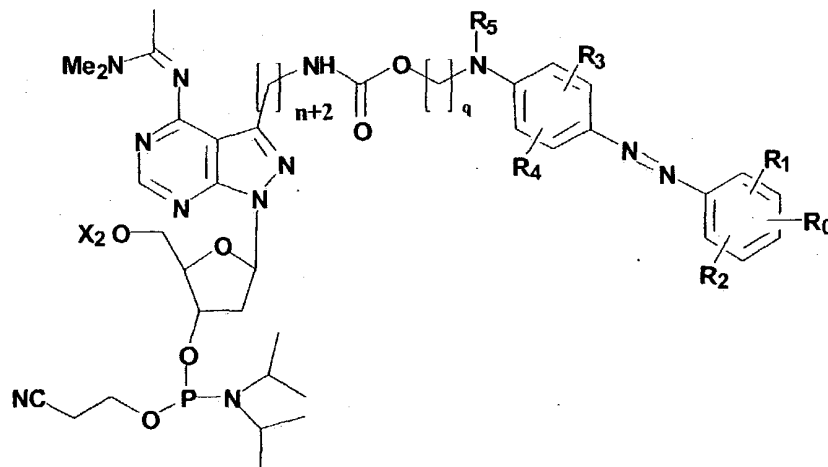
37. A phosphoramidite reagent in accordance with Claim 34 that has the formula designated PA-5.

38. A phosphoramidite reagent in accordance with Claim 37 where R_{10} is $OC(O)CH(CH_3)_2$, R_{11} is H and h is 3.

39. A phosphoramidite reagent in accordance with Claim 34 that has the formula designated PA-6.

40. A phosphoramidite reagent in accordance with Claim 39 where R_{15} is methyl and R_{16} is *n*-propyl.

41. A phosphoramidite reagent for preparing an oligonucleotide-fluorophore-quencher conjugate, the reagent having the formula



wherein R_0 , R_1 , R_2 , R_3 and R_4 are independently -H, halogen, $-O(CH_2)_{n^*}CH_3$, $-(CH_2)_{n^*}CH_3$ where $n^* = 0$ to 5, $-NO_2$, $-SO_3$, $-N[(CH_2)_{n^*}CH_3]_2$ where $n^* = 0$ to 5 or $-CN$, and $R_5 = -H$ or $-(CH_2)_{n''}CH_3$ where $n'' = 0$ to 5;

n is 1 to 10;

q is 1 to 20, and

X_2 is H or dimethoxytrityl, methoxytrityl, trityl or an acid labile blocking group.

42. A phosphoramidite reagent in accordance with Claim 41 where R_0 is H, R_1 is NO_2 in the 4 position of the benzene nucleus, R_2 is Cl in the 2 position of the benzene nucleus, and R_3 and R_4 are hydrogen, R_5 is ethyl, n is 1 and q is 2.

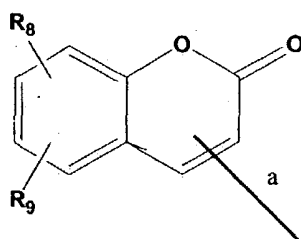
43. An oligonucleotide conjugate having the formula

FL-ODN-MGB

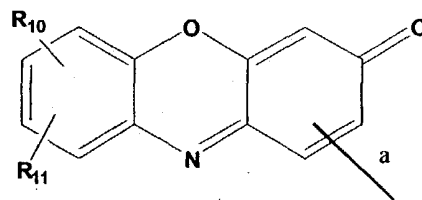
where ODN is an oligonucleotide or nucleic acid;

MGB is minor groove binder moiety covalently attached to the ODN moiety or to the quencher moiety through a linker having the length of 0 to approximately 30 atoms;

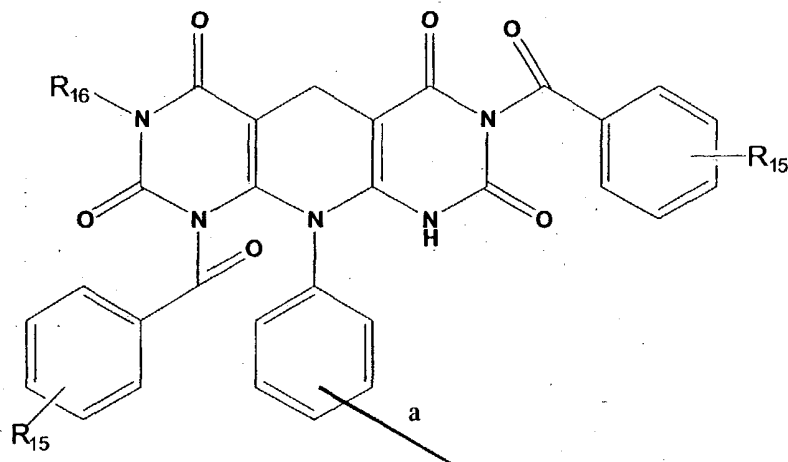
FL is a fluorophore covalently attached to the ODN through a linker having the length of 0 to approximately 30 atoms, said fluorophore moiety having the structure selected from the group designated FL-1, FL-2 and FL-3,



FL-1



FL-2



FL-3

wherein R_8 and R_9 independently are H, halogen, $-\text{NO}_2$, $-\text{SO}_3$, $-\text{C}(=\text{O})\text{NH}_2$, or $-\text{CN}$; $-\text{OR}_{nn}$, $-\text{SR}_{nn}$, $-\text{OR}_{nn}$, $-\text{NHR}_{nn}$, $-\text{N}[\text{R}_{nn}]_2$ where R_{nn} is independently H, an alkyl group of 1 to 10 carbons or an alkanoyl group of 1 to 10 carbons;

R_{10} and R_{11} independently are H, $-\text{CN}$, $-\text{OR}_{12}$, $-\text{N}(\text{R}_{12})_2$, halogen, $-\text{O}(\text{CH}_2)_n\text{CH}_3$, $-(\text{CH}_2)_n\text{CH}_3$, $-\text{NO}_2$, $-\text{SO}_3$, $-\text{C}(=\text{O})\text{NH}_2$, $-\text{N}[(\text{CH}_2)_n\text{CH}_3]_2$ where $n=0$ to 5, or R_{12} is alkyl of 1 to 10 carbons alkanoyl of 1 to 10 carbons,;

R_{15} is H or alkyl of 1 to 10 carbons;

R_{16} is alkyl of 1 to 10 carbons, and

the valence bond designated **a** symbolizes covalent attachment of the fluorophore to the linker.

44. An oligonucleotide conjugate in accordance with Claim 43 where the fluorophore has the formula designated FL-1.

45. An oligonucleotide conjugate in accordance with Claim 44 where R_8 is $-\text{OC}(\text{O})\text{CH}(\text{CH}_3)_2$ and R_9 is H.

46. An oligonucleotide conjugate in accordance with Claim 43 where the fluorophore has the formula designated FL-2.

47. An oligonucleotide conjugate in accordance with Claim 46 where R_{10} is $\text{OC}(\text{O})\text{CH}(\text{CH}_3)_2$ and R_{11} is H.

48. An oligonucleotide conjugate in accordance with Claim 43 where the fluorophore has the formula designated FL-3.

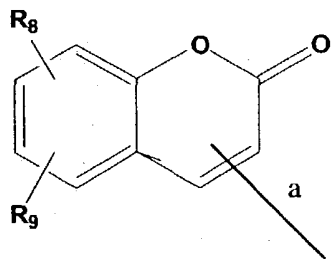
49. An oligonucleotide conjugate in accordance with Claim 49 where R_{15} is methyl and R_{16} is *n*-propyl.

50. An oligonucleotide conjugate having the formula

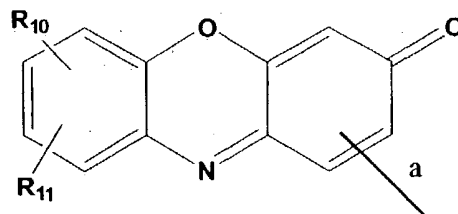
FL-ODN

where ODN is an oligonucleotide or nucleic acid;

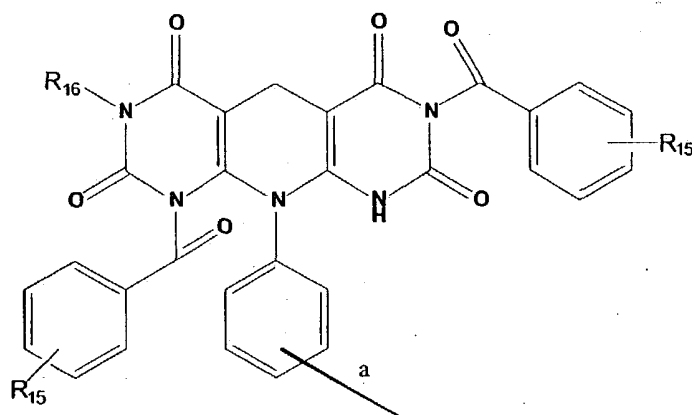
FL is a fluorophore covalently attached to the ODN through a linker having the length of 0 to approximately 30 atoms, said fluorophore moiety having the structure selected from the group designated FL-1, FL-2 and FL-3,



FL-1



FL-2



FL-3

wherein R_8 and R_9 independently are H, halogen, $-\text{NO}_2$, $-\text{SO}_3$, $-\text{C}(=\text{O})\text{NH}_2$, or $-\text{CN}$; $-\text{OR}_{nn}$, $-\text{SR}_{nn}$, $-\text{OR}_{nn}$, $-\text{NHR}_{nn}$, $-\text{N}[\text{R}_{nn}]_2$ where R_{nn} is independently H, an alkyl group of 1 to 10 carbons or an alkanoyl group of 1 to 10 carbons;

R_{10} and R_{11} independently are H, $-\text{CN}$, $-\text{OR}_{12}$, $-\text{N}(\text{R}_{12})_2$, halogen, $-\text{O}(\text{CH}_2)_n\text{CH}_3$, $-(\text{CH}_2)_n\text{CH}_3$, $-\text{NO}_2$, $-\text{SO}_3$, $-\text{C}(=\text{O})\text{NH}_2$, $-\text{N}[(\text{CH}_2)_n\text{CH}_3]_2$ where $n = 0$ to 5, or R_{12} is alkyl of 1 to 10 carbons alkanoyl of 1 to 10 carbons,;

R_{15} is H or alkyl of 1 to 10 carbons;

R_{16} is alkyl of 1 to 10 carbons, and

the valence bond designated **a** symbolizes covalent attachment of the fluorophore to the linker.

51. An oligonucleotide conjugate in accordance with Claim 50 where the fluorophore has the formula designated FL-1.

52. An oligonucleotide conjugate in accordance with Claim 51 where R_8 is $OC(O)CH(CH_3)_2$ and R_9 is H.

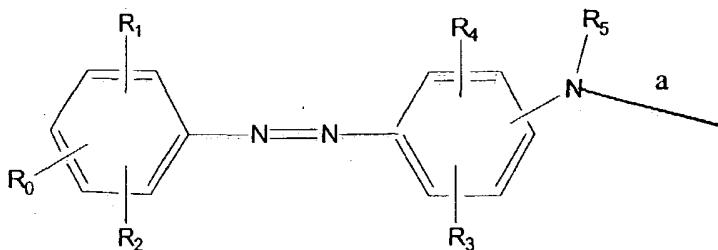
53. An oligonucleotide conjugate in accordance with Claim 50 where the fluorophore has the formula designated FL-2.

54. An oligonucleotide conjugate in accordance with Claim 53 where R_{10} is $OC(O)CH(CH_3)_2$ and R_{11} is H.

55. An oligonucleotide conjugate in accordance with Claim 50 where the fluorophore has the formula designated FL-3.

56. An oligonucleotide conjugate in accordance with Claim 55 where R_{15} is methyl and R_{16} is *n*-propyl.

57. A method for hybridizing nucleic acids, comprising the steps of:
(a) providing a first nucleic acid and a second nucleic acid,
(b) incubating the nucleic acids under hybridization conditions, and
(c) identifying hybridized nucleic acids; wherein at least one of the nucleic acids comprises a **FL-nucleic-acid-Q** conjugate where **FL** is a fluorophore moiety covalently attached to the nucleic acid through a linker having the length of 0 to approximately 30 atoms,, and **Q** is a quencher moiety covalently attached to the nucleic acid through a linker having the length of 0 to approximately 30 atoms, the quencher moiety having the structure

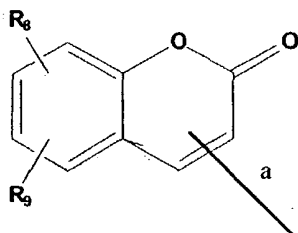


where R_0 , R_1 , R_2 , R_3 and R_4 are independently -H, halogen, $-O(CH_2)_nCH_3$, $-(CH_2)_nCH_3$ where $n = 0$ to 5 , $-NO_2$, $-SO_3$, $-N[(CH_2)_nCH_3]_2$ where $n' = 0$ to 5 or $-CN$, and $R_5 = -H$ or $-(CH_2)_{n''}CH_3$ where $n'' = 0$ to 5 , and where the quencher moiety is attached to the linker through the valence bond designated a.

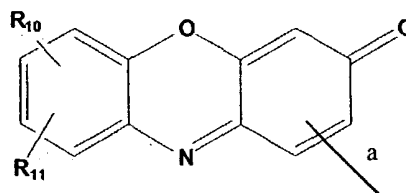
58. A method in accordance with Claim 57 where in the formula **Q** of the quencher moiety R_0 is H, R_1 is NO_2 in the 4 position of the benzene nucleus, R_2 is Cl in the 2 position of the benzene nucleus, and R_3 and R_4 are hydrogen and R_5 is ethyl.

59. A method for hybridizing nucleic acids, comprising the steps of:
 (a) providing a first nucleic acid and a second nucleic acid,
 (b) incubating the nucleic acids under hybridization conditions, and
 (c) identifying hybridized nucleic acids; wherein at least one of the nucleic acids comprises a **FL-nucleic-acid-Q** conjugate where **Q** is a quencher moiety covalently attached to the nucleic acid through a linker having the length of 0 to approximately 30 atoms, and

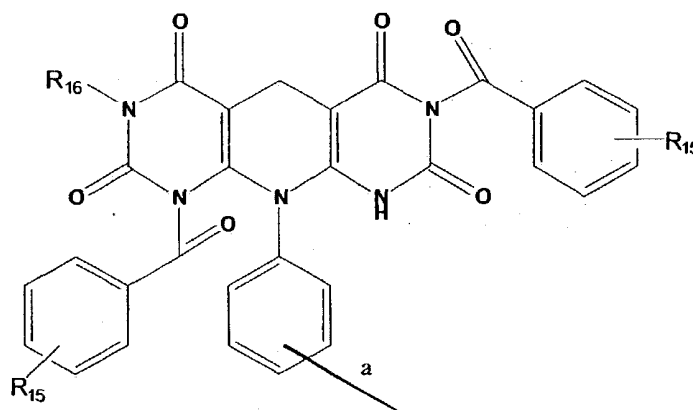
wherein FL is a fluorophore covalently attached to the ODN through a linker having the length of 0 to approximately 30 atoms, said fluorophore moiety having the structure selected from the group designated FL-1, FL-2 and FL-3,



FL-1



FL-2



FL-3

wherein R_8 and R_9 independently are H, halogen, $-\text{NO}_2$, $-\text{SO}_3$, $-\text{C}(=\text{O})\text{NH}_2$, or $-\text{CN}$; $-\text{OR}_{nn}$, $-\text{SR}_{nn}$, $-\text{OR}_{nn}$, $-\text{NHR}_{nn}$, $-\text{N}[\text{R}_{nn}]_2$ where R_{nn} is independently H, an alkyl group of 1 to 10 carbons or an alkanoyl group of 1 to 10 carbons;

R_{10} and R_{11} independently are H, $-\text{CN}$, $-\text{OR}_{12}$, $-\text{N}(\text{R}_{12})_2$, halogen, $-\text{O}(\text{CH}_2)_n\text{CH}_3$, $-(\text{CH}_2)_n\text{CH}_3$, $-\text{NO}_2$, $-\text{SO}_3$, $-\text{C}(=\text{O})\text{NH}_2$, $-\text{N}[(\text{CH}_2)_n\text{CH}_3]_2$ where $n=0$ to 5, or R_{12} is alkyl of 1 to 10 carbons alkanoyl of 1 to 10 carbons,;

R_{15} is H or alkyl of 1 to 10 carbons;

R_{16} is alkyl of 1 to 10 carbons, and

the valence bond designated **a** symbolizes covalent attachment of the fluorophore to the linker.

60. A method in accordance with Claim 59 where the fluorophore has the formula designated FL-1.

61. A method in accordance with Claim 60 where R_8 is $OC(O)CH(CH_3)_2$ and R_9 is H.

62. A method in accordance with Claim 59 where the fluorophore has the formula designated FL-2.

63. A method in accordance with Claim 62 where R_{10} is $OC(O)CH(CH_3)_2$ and R_{11} is H.

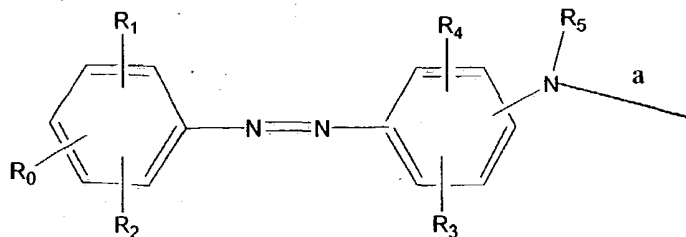
64. A method in accordance with Claim 59 where the fluorophore has the formula designated FL-3.

65. A method in accordance with Claim 64 where R_{15} is methyl and R_{16} is *n*-propyl.

66. A method for discriminating between polynucleotides which differ by a single nucleotide, the method comprising the following steps:

- (a) providing a polynucleotide comprising a target sequence,
- (b) providing at least two **FL-ODN-Q** conjugates, wherein ODN represents an oligonucleotide moiety, one of the at least two **FL-ODN-Q** conjugates has a sequence that is perfectly complementary to the target sequence and at least one other of the **FL-ODN-Q** conjugates has a single-nucleotide mismatch with the target sequence;
- (c) separately incubating each of the **FL-ODN-Q** conjugates with the polynucleotide under hybridization conditions; and
- (d) determining the hybridization strength between each of the **FL-ODN-Q** and the polynucleotide, wherein **FL** is a fluorophore moiety covalently attached to the nucleic acid through a linker having the length of 0 to approximately 30 atoms, and **Q** is a quencher moiety covalently attached to

the nucleic acid through a linker having the length of 0 to approximately 30 atoms, the quencher moiety having the structure



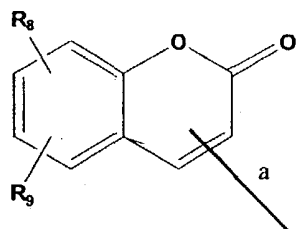
where R_0 , R_1 , R_2 , R_3 and R_4 are independently -H, halogen, $-O(CH_2)_nCH_3$, $-(CH_2)_nCH_3$ where $n = 0$ to 5, $-NO_2$, $-SO_3$, $-N[(CH_2)_nCH_3]_2$ where $n' = 0$ to 5 or $-CN$, and $R_5 = -H$ or $-(CH_2)_nCH_3$ where $n'' = 0$ to 5, and where the quencher moiety is attached to the linker through the valence bond designated a.

67. A method in accordance with Claim 66 where in the formula of the quencher moiety Q R_0 is H, R_1 is NO_2 in the 4 position of the benzene nucleus, R_2 is Cl in the 2 position of the benzene nucleus, and R_3 and R_4 are hydrogen and R_5 is ethyl.

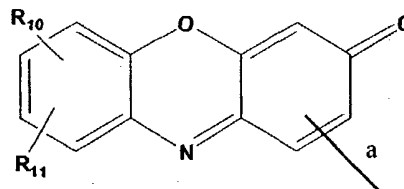
68. A method for discriminating between polynucleotides which differ by a single nucleotide, the method comprising the following steps:

- (a) providing a polynucleotide comprising a target sequence,
- (b) providing at least two **FL-ODN-Q** conjugates, wherein ODN represents an oligonucleotide moiety, one of the at least two **FL-ODN-Q** conjugates has a sequence that is perfectly complementary to the target sequence and at least one other of the **FL-ODN-Q** conjugates has a single-nucleotide mismatch with the target sequence;
- (c) separately incubating each of the **FL-ODN-Q** conjugates with the polynucleotide under hybridization conditions; and
- (d) determining the hybridization strength between each of the **FL-ODN-Q** and the polynucleotide, wherein Q is a quencher moiety covalently

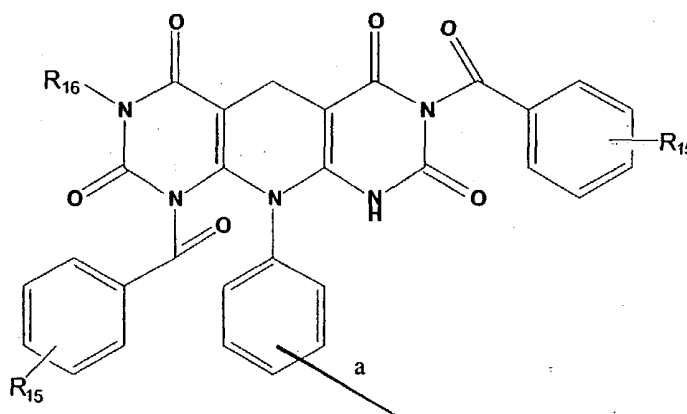
attached to the nucleic acid through a linker having the length of 0 to approximately 30 atoms, and **FL** is a fluorophore moiety covalently attached to the nucleic acid through a linker having the length of 0 to approximately 30 atoms,, and the fluorophore moiety having the structure selected from the group designated FL-1, FL-2 and FL-3,



FL-1



FL-2



FL-3

wherein R_8 and R_9 independently are H, halogen, $-\text{NO}_2$, $-\text{SO}_3$, $-\text{C}(=\text{O})\text{NH}_2$, or $-\text{CN}$; $-\text{OR}_{nn}$, $-\text{SR}_{nn}$, $-\text{OR}_{nn}$, $-\text{NHR}_{nn}$, $-\text{N}[\text{R}_{nn}]_2$ where R_{nn} is independently H, an alkyl group of 1 to 10 carbons or an alkanoyl group of 1 to 10 carbons;

R_{10} and R_{11} independently are H, $-\text{CN}$, $-\text{OR}_{12}$, $-\text{N}(\text{R}_{12})_2$, halogen, $-\text{O}(\text{CH}_2)_n\text{CH}_3$, $-(\text{CH}_2)_n\text{CH}_3$, $-\text{NO}_2$, $-\text{SO}_3$, $-\text{C}(=\text{O})\text{NH}_2$, $-\text{N}[(\text{CH}_2)_n\text{CH}_3]_2$ where $n = 0$ to 5, or R_{12} is alkyl of 1 to 10 carbons alkanoyl of 1 to 10 carbons,;

R_{15} is H or alkyl of 1 to 10 carbons;

R_{16} is alkyl of 1 to 10 carbons, and
the valence bond designated **a** symbolizes covalent attachment of the fluorophore to the linker.

69. A method in accordance with Claim 68 where the fluorophore has the formula designated FL-1.

70. A method in accordance with Claim 69 where R_8 is $OC(O)CH(CH_3)_2$ and R_9 is H.

71. A method in accordance with Claim 68 where the fluorophore has the formula designated FL-2.

72. A method in accordance with Claim 71 where R_{10} is $OC(O)CH(CH_3)_2$ and R_{11} is H.

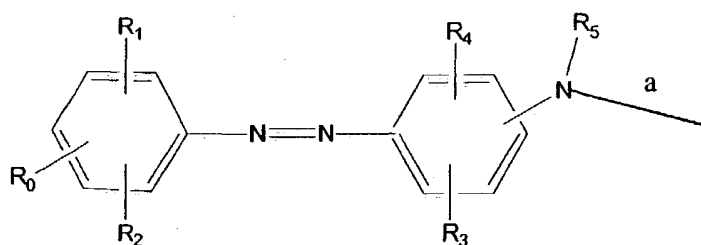
73. A method in accordance with Claim 68 where the fluorophore has the formula designated FL-3.

74. A method in accordance with Claim 73 where R_{15} is methyl and R_{16} is *n*-propyl.

75. A method for hybridizing nucleic acids, comprising the steps of:
(a) providing a first nucleic acid and a second nucleic acid,
(b) incubating the nucleic acids under hybridization conditions, and
(c) identifying hybridized nucleic acids;

wherein at least one of the nucleic acids comprises a **FL-nucleic-acid-Q-MGB** conjugate where **FL** is a fluorophore moiety covalently attached to the nucleic acid through a linker having the length of 0 to approximately 30 atoms, MGB is minor groove binder moiety covalently attached to the ODN

moiety or to the quencher moiety through a linker having the length of 0 to approximately 30 atoms and **Q** is a quencher moiety covalently attached to the nucleic acid through a linker having the length of 0 to approximately 30 atoms, the quencher moiety having the structure

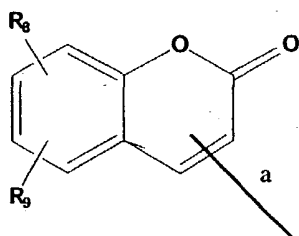


where R_0 , R_1 , R_2 , R_3 and R_4 are independently -H, halogen, $-O(CH_2)_nCH_3$, $-(CH_2)_nCH_3$ where $n=0$ to 5, $-NO_2$, $-SO_3$, $-N[(CH_2)_{n'}CH_3]_2$ where $n'=0$ to 5 or $-CN$, and $R_5 = -H$ or $-(CH_2)_{n''}CH_3$ where $n''=0$ to 5, and where the quencher moiety is attached to the linker through the valence bond designated a.

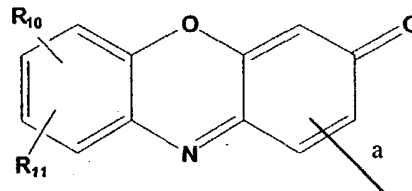
76. A method for hybridizing nucleic acids, comprising the steps of:

- (a) providing a first nucleic acid and a second nucleic acid,
- (b) incubating the nucleic acids under hybridization conditions, and
- (c) identifying hybridized nucleic acids;

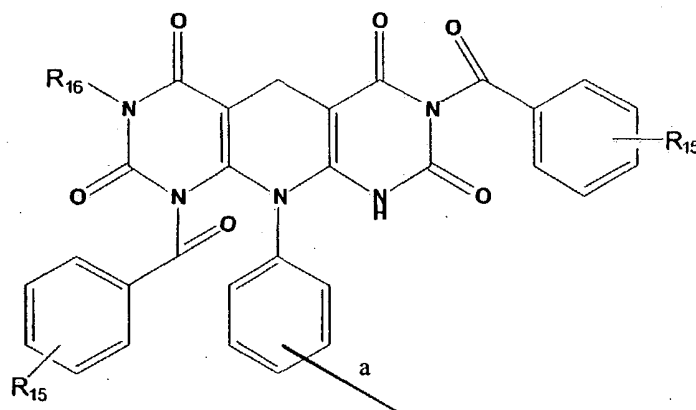
wherein at least one of the nucleic acids comprises a **FL-ODN-Q-MGB** conjugate where ODN is a nucleic acid or modified nucleic acid, MGB is minor groove binder moiety covalently attached to the ODN moiety or to the quencher moiety through a linker having the length of 0 to approximately 30 atoms, **Q** is a quencher moiety covalently attached to the ODN through a linker having the length of 0 to approximately 30 atoms, and **FL** is a fluorophore moiety covalently attached to the nucleic acid through a linker having the length of 0 to approximately 30 atoms, and the fluorophore moiety having the structure selected from the group designated FL-1, FL-2 and FL-3,



FL-1



FL-2



FL-3

wherein R_8 and R_9 independently are H, halogen, $-\text{NO}_2$, $-\text{SO}_3$, $-\text{C}(=\text{O})\text{NH}_2$, or $-\text{CN}$; $-\text{OR}_{nn}$, $-\text{SR}_{nn}$, $-\text{OR}_{nn}$, $-\text{NHR}_{nn}$, $-\text{N}[\text{R}_{nn}]_2$ where R_{nn} is independently H, an alkyl group of 1 to 10 carbons or an alkanoyl group of 1 to 10 carbons;

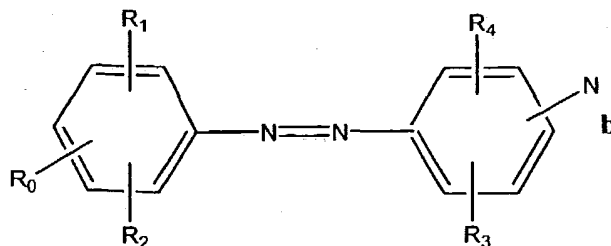
R_{10} and R_{11} independently are H, $-\text{CN}$, $-\text{OR}_{12}$, $-\text{N}(\text{R}_{12})_2$, halogen, $-\text{O}(\text{CH}_2)_n\text{CH}_3$, $-(\text{CH}_2)_n\text{CH}_3$, $-\text{NO}_2$, $-\text{SO}_3$, $-\text{C}(=\text{O})\text{NH}_2$, $-\text{N}[(\text{CH}_2)_n\text{CH}_3]_2$ where $n=0$ to 5, or R_{12} is alkyl of 1 to 10 carbons alkanoyl of 1 to 10 carbons,;

R_{15} is H or alkyl of 1 to 10 carbons;

R_{16} is alkyl of 1 to 10 carbons, and

the valence bond designated a symbolizes covalent attachment of the fluorophore to the linker; and

Q comprises a diazo moiety having the formula:

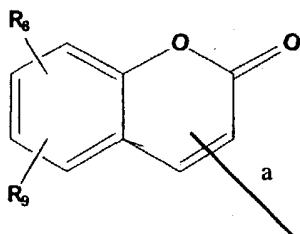


wherein covalent attachment to the linker is through the nitrogen atom designated as **b**.

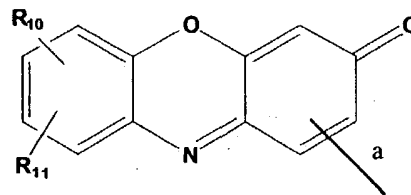
77. A method for hybridizing nucleic acids, comprising the steps of:

- (a) providing a first nucleic acid and a second nucleic acid,
- (b) incubating the nucleic acids under hybridization conditions, and
- (c) identifying hybridized nucleic acids;

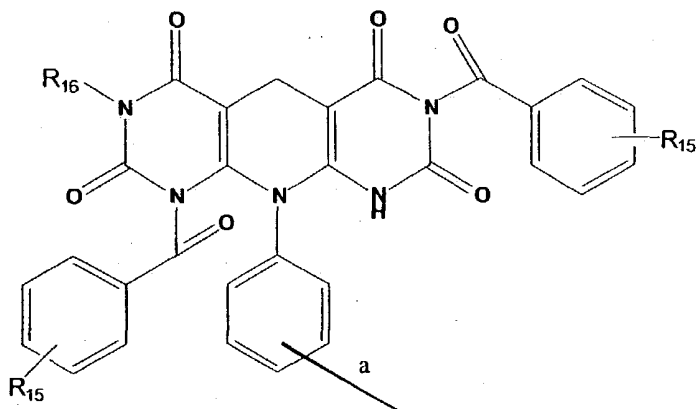
wherein at least one of the nucleic acids comprises a **FL-ODN-Q-MGB** conjugate where ODN is a nucleic acid or modified nucleic acid, MGB is minor groove binder moiety covalently attached to the ODN moiety or to the quencher moiety through a linker having the length of 0 to approximately 30 atoms, **Q** is a quencher moiety covalently attached to the ODN through a linker having the length of 0 to approximately 30 atoms, and **FL** is a fluorophore moiety covalently attached to the nucleic acid through a linker having the length of 0 to approximately 30 atoms,, and the fluorophore moiety having the structure selected from the group designated FL-1, FL-2 and FL-3,



FL-1



FL-2



FL-3

wherein R_8 and R_9 independently are H, halogen, $-\text{NO}_2$, $-\text{SO}_3$, $-\text{C}(=\text{O})\text{NH}_2$, or $-\text{CN}$; $-\text{OR}_{nn}$, $-\text{SR}_{nn}$, $-\text{OR}_{nn}$, $-\text{NHR}_{nn}$, $-\text{N}[\text{R}_{nn}]_2$ where R_{nn} is independently H, an alkyl group of 1 to 10 carbons or an alkanoyl group of 1 to 10 carbons;

R_{10} and R_{11} independently are H, $-\text{CN}$, $-\text{OR}_{12}$, $-\text{N}(\text{R}_{12})_2$, halogen, $-\text{O}(\text{CH}_2)_n\text{CH}_3$, $-(\text{CH}_2)_n\text{CH}_3$, $-\text{NO}_2$, $-\text{SO}_3$, $-\text{C}(=\text{O})\text{NH}_2$, $-\text{N}[(\text{CH}_2)_n\text{CH}_3]_2$ where $n = 0$ to 5, or R_{12} is alkyl of 1 to 10 carbons alkanoyl of 1 to 10 carbons,;

R_{15} is H or alkyl of 1 to 10 carbons;

R_{16} is alkyl of 1 to 10 carbons, and

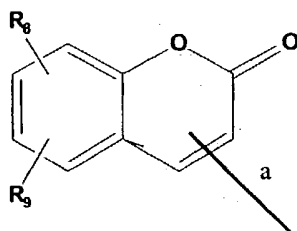
the valence bond designated **a** symbolizes covalent attachment of the fluorophore to the linker.

78. A method for hybridizing nucleic acids, comprising the steps of:

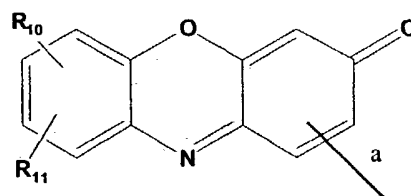
- (a) providing a first nucleic acid and a second nucleic acid,
- (b) incubating the nucleic acids under hybridization conditions, and
- (c) identifying hybridized nucleic acids;

wherein at least one of the nucleic acids comprises a **FL-ODN-Q** conjugate where ODN is a nucleic acid or modified nucleic acid, **Q** is a quencher moiety covalently attached to the ODN through a linker having the length of 0 to approximately 30 atoms, and **FL** is a fluorophore moiety covalently attached to the nucleic acid through a linker having the length of 0

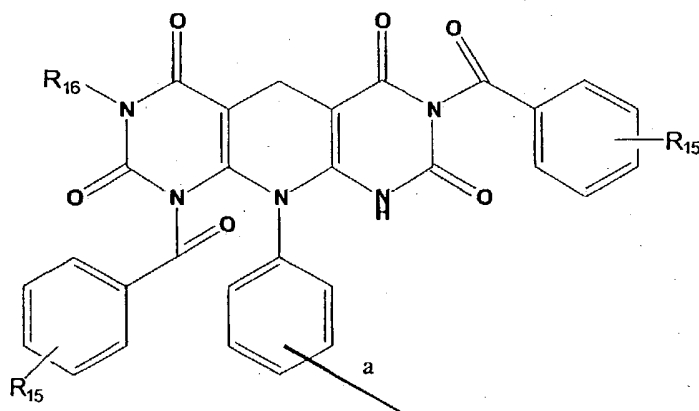
to approximately 30 atoms,, and the fluorophore moiety having the structure selected from the group designated FL-1, FL-2 and FL-3,



FL-1



FL-2



FL-3

wherein R_8 and R_9 independently are H, halogen, $-\text{NO}_2$, $-\text{SO}_3$, $-\text{C}(=\text{O})\text{NH}_2$, or $-\text{CN}$; $-\text{OR}_{nn}$, $-\text{SR}_{nn}$, $-\text{OR}_{nn}$, $-\text{NHR}_{nn}$, $-\text{N}[\text{R}_{nn}]_2$ where R_{nn} is independently H, an alkyl group of 1 to 10 carbons or an alkanoyl group of 1 to 10 carbons;

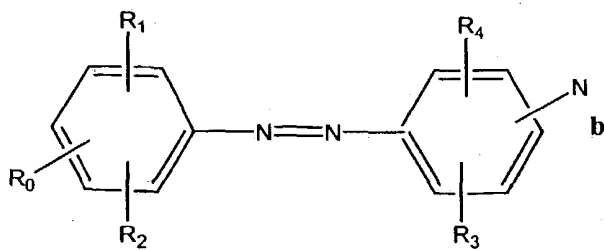
R_{10} and R_{11} independently are H, $-\text{CN}$, $-\text{OR}_{12}$, $-\text{N}(\text{R}_{12})_2$, halogen, $-\text{O}(\text{CH}_2)_n\text{CH}_3$, $-(\text{CH}_2)_n\text{CH}_3$, $-\text{NO}_2$, $-\text{SO}_3$, $-\text{C}(=\text{O})\text{NH}_2$, $-\text{N}[(\text{CH}_2)_n\text{CH}_3]_2$ where $n = 0$ to 5, or R_{12} is alkyl of 1 to 10 carbons alkanoyl of 1 to 10 carbons,;

R_{15} is H or alkyl of 1 to 10 carbons;

R_{16} is alkyl of 1 to 10 carbons, and

the valence bond designated **a** symbolizes covalent attachment of the fluorophore to the linker; and

Q comprises a diazo moiety having the formula:



wherein covalent attachment to the linker is through the nitrogen atom designated as **b**.